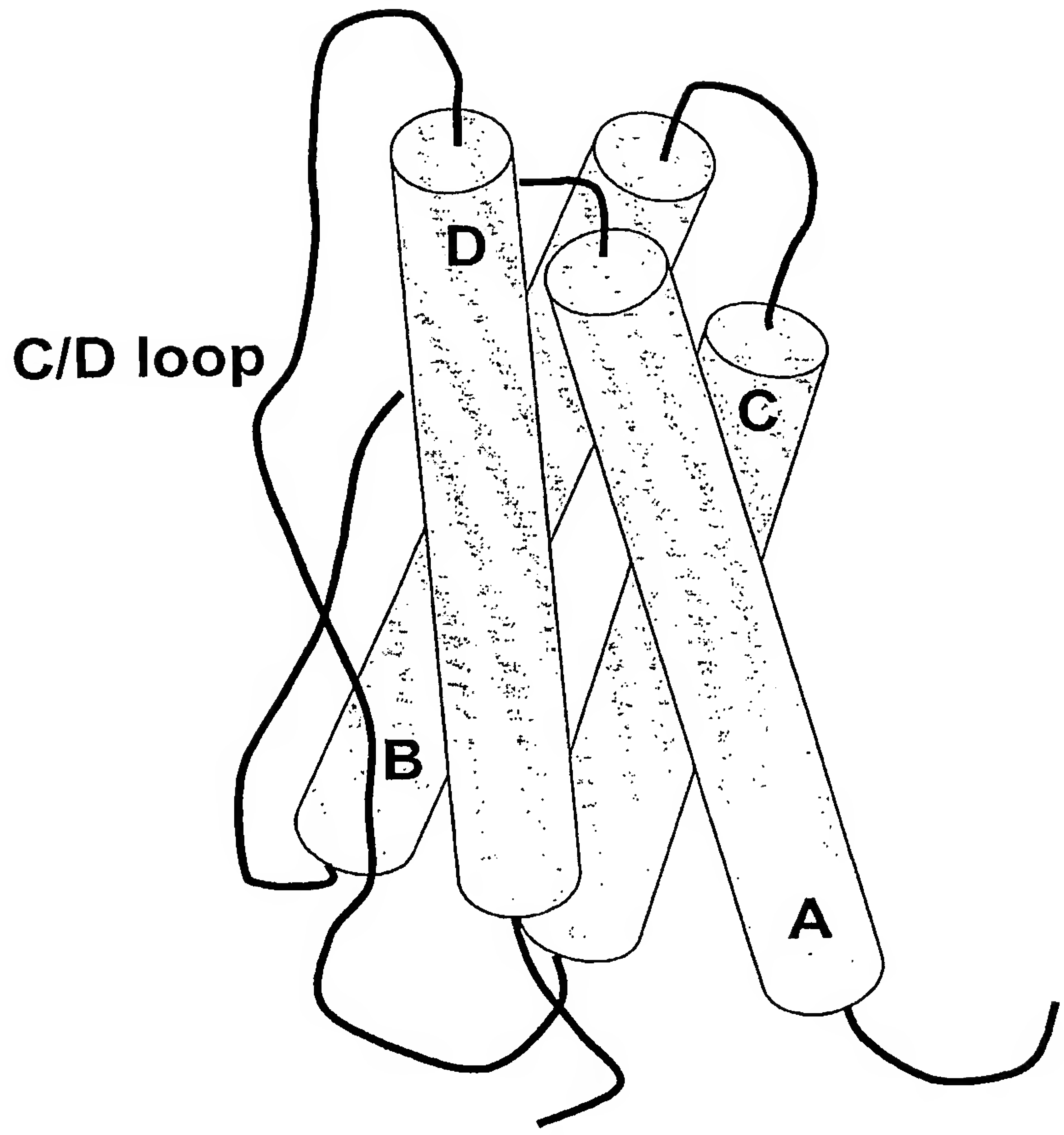
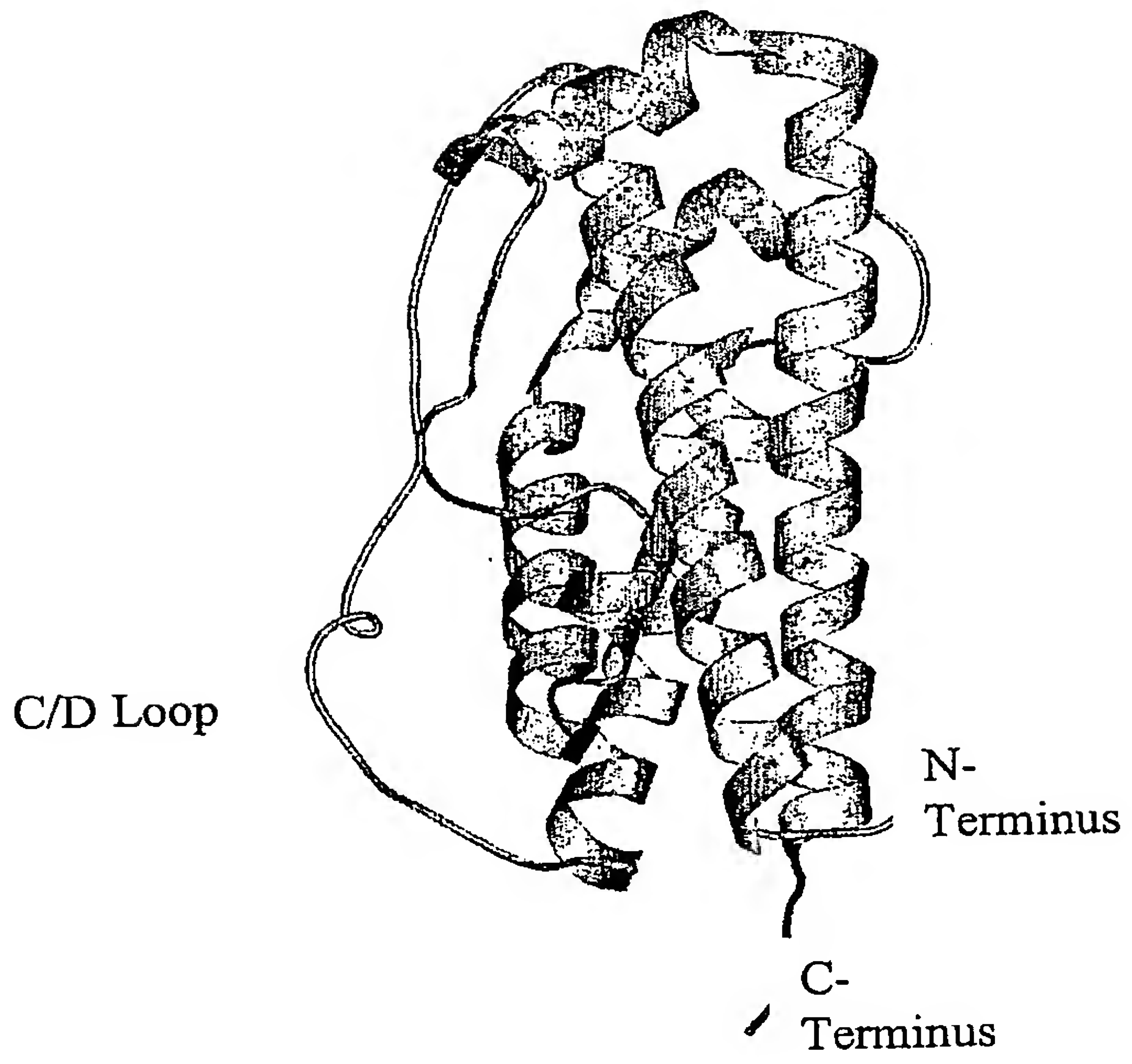
Figure 1


C/D loop
D
C
B
A

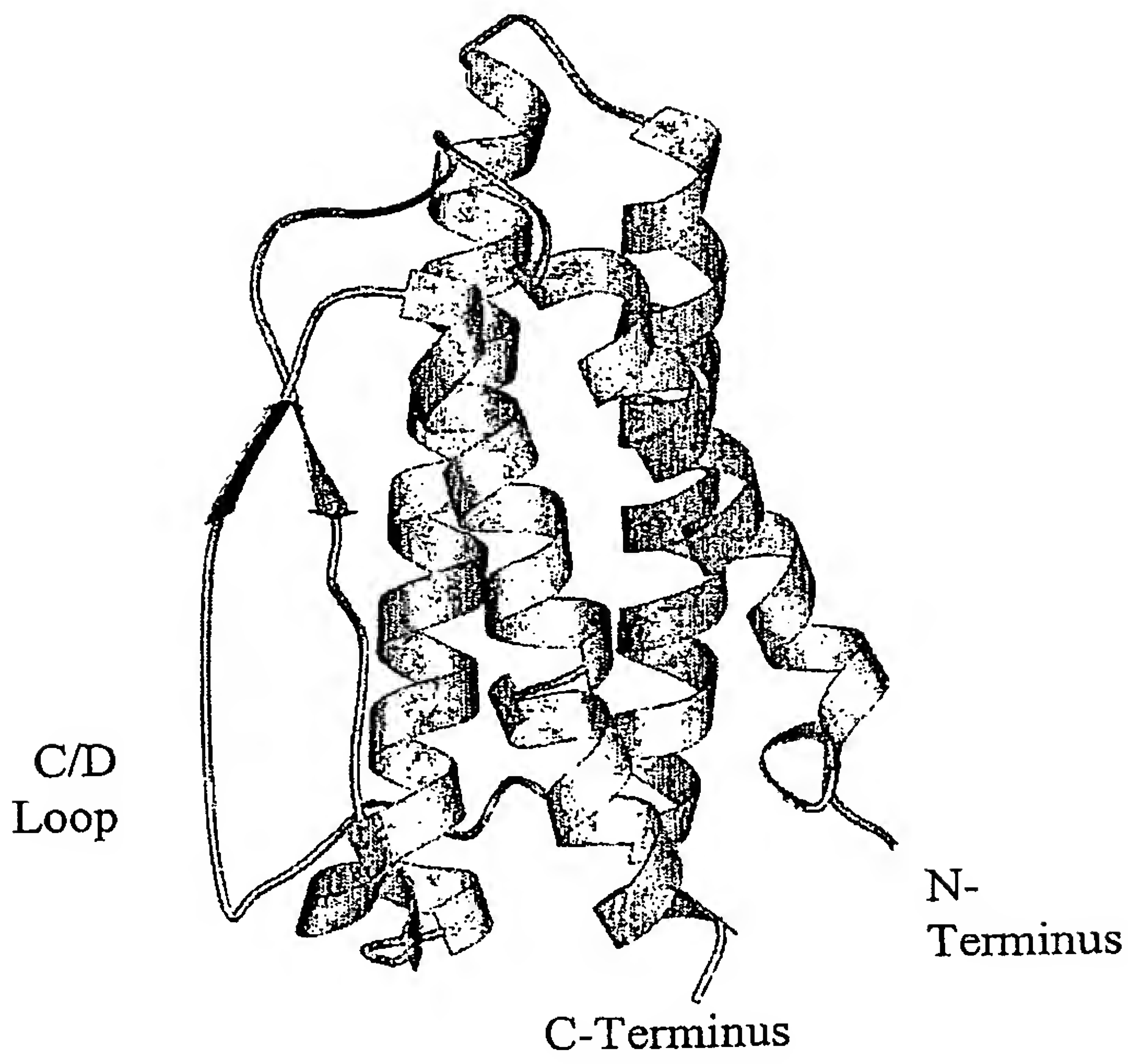
A Four Helical Bundle Protein

Figure 2


C/D Loop
N-
Terminus
C-
Terminus

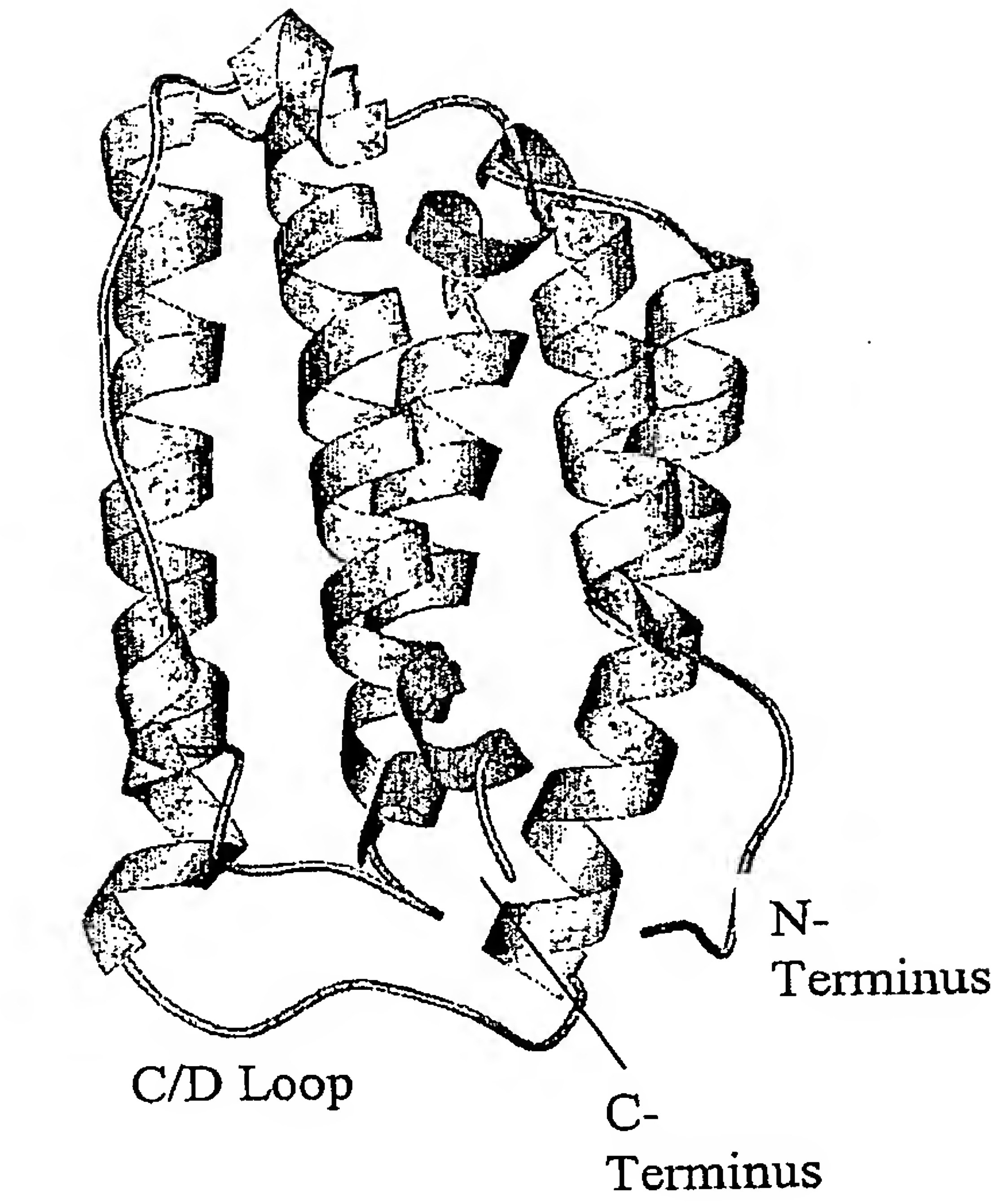
hGH

Figure 3


C/D
Loop
N-
Terminus
C-Terminus

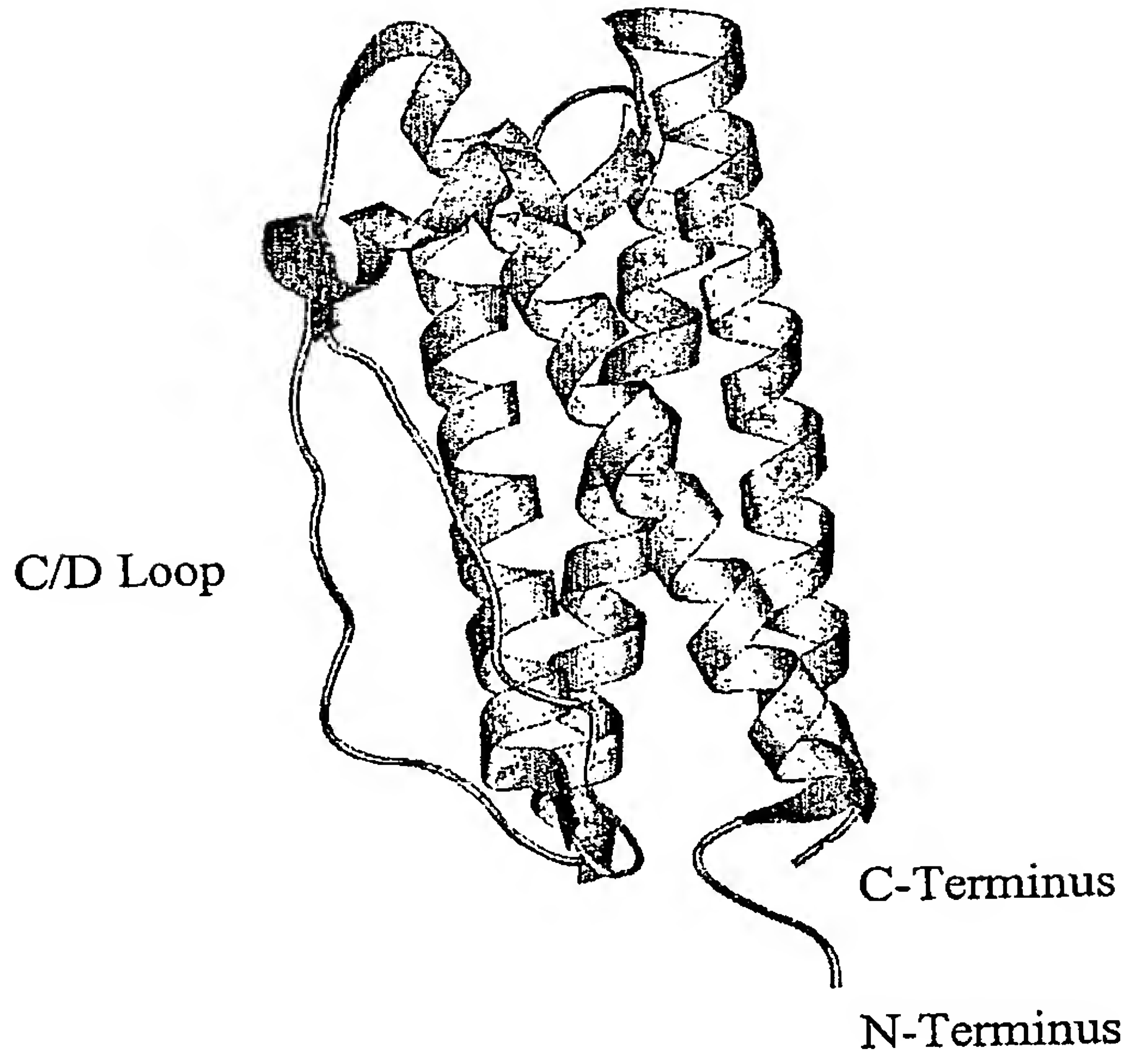
EPO

Figure 4


N-
Terminus
C/D Loop
C-
Terminus

IFNα-2

Figure 5


C/D Loop
C-Terminus
N-Terminus

G-CSF

Figure 6

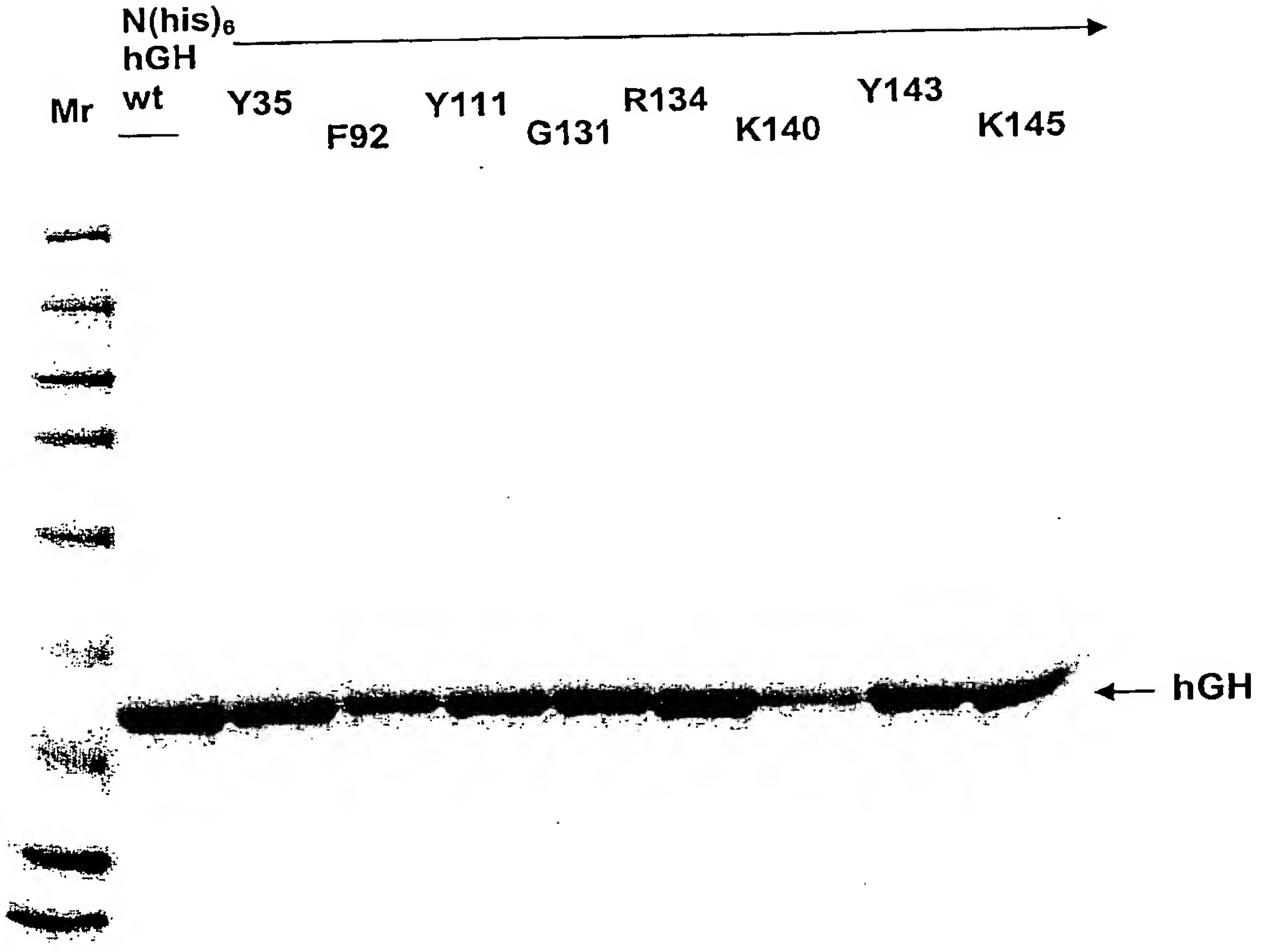

N(his)6
hGH
wt
Mr
Y35
F92
Y111
G131
R134
K140
Y143
K145
hGH

Figure 7, Panel A:

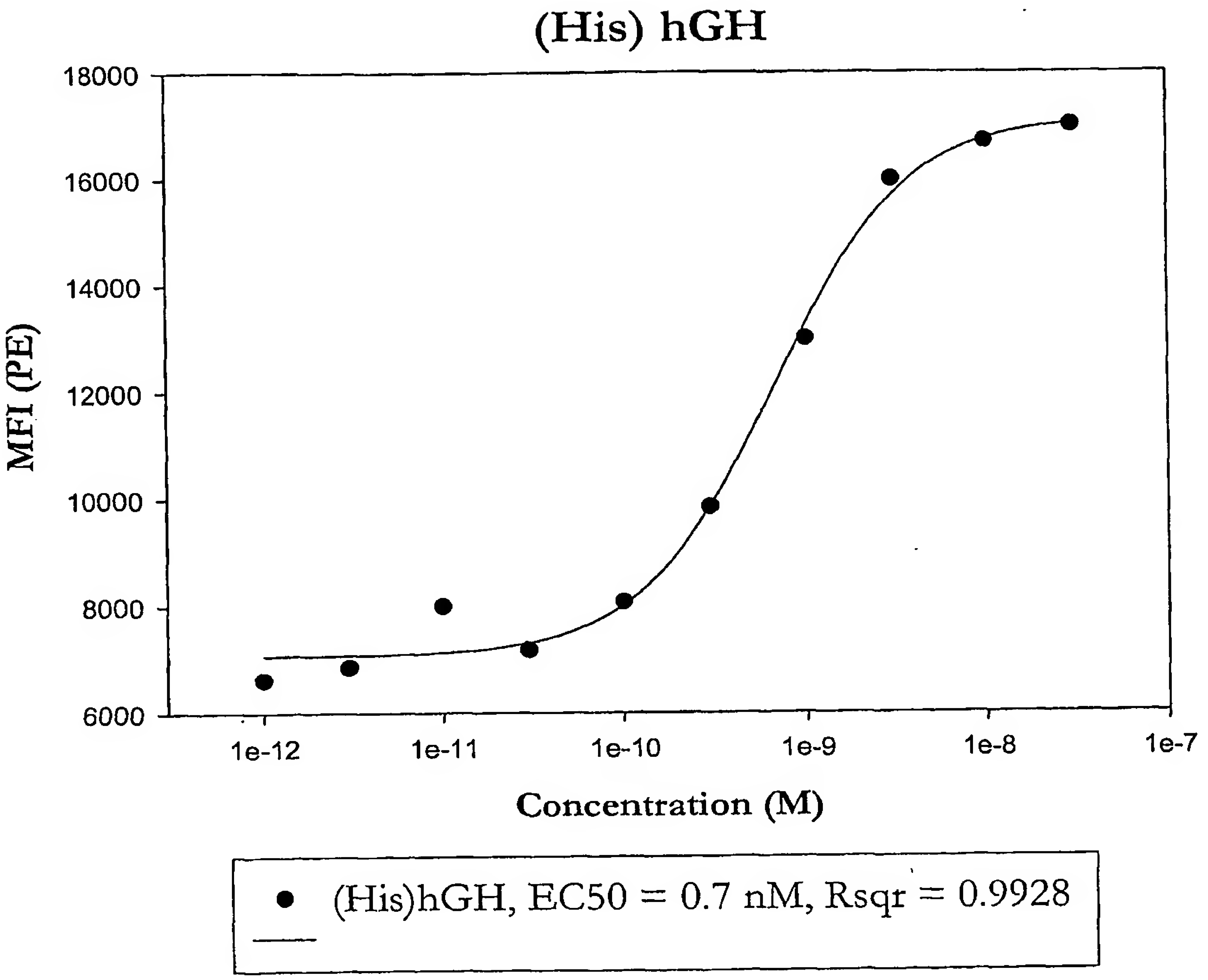


Figure 7, Panel B:

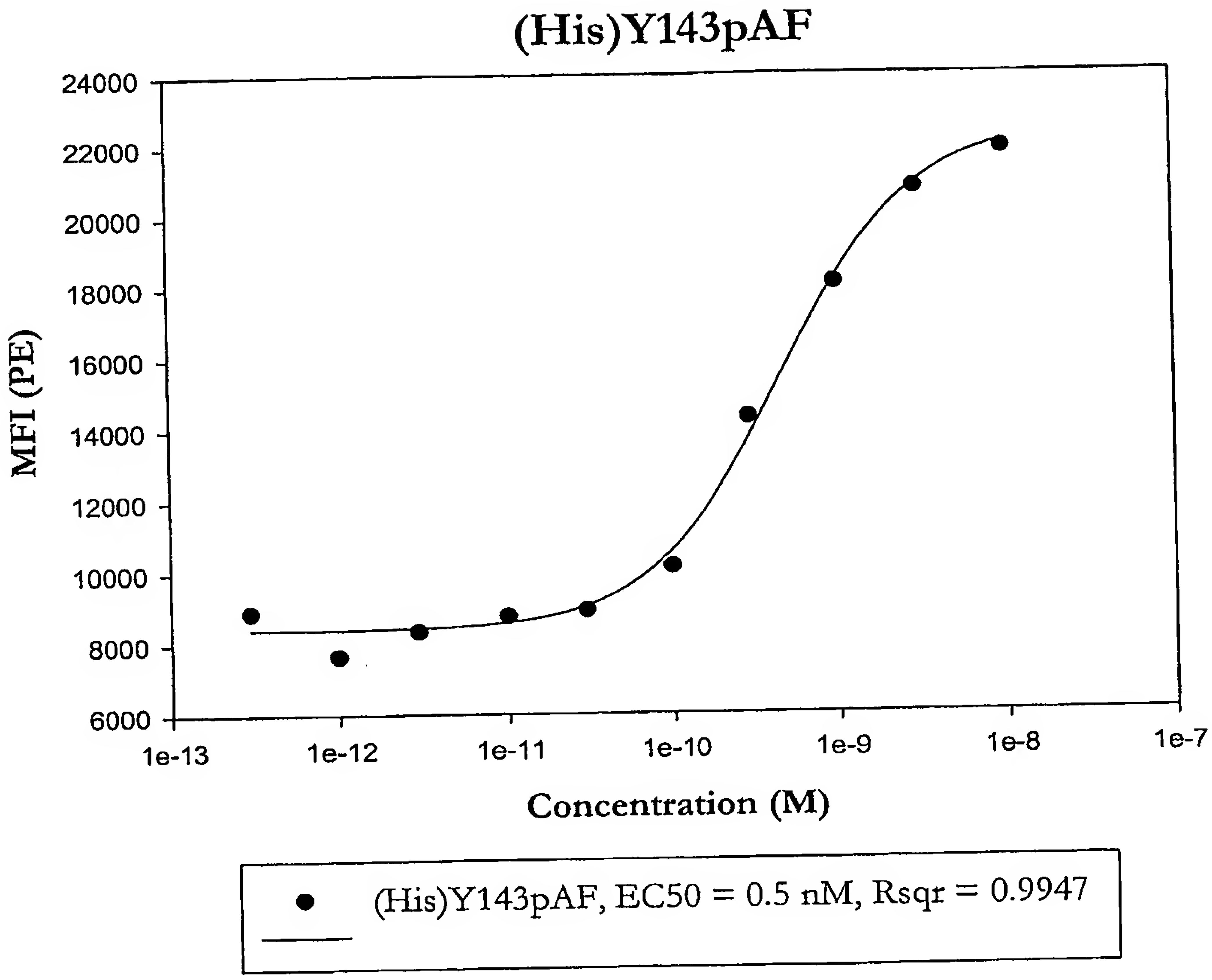


Figure 8

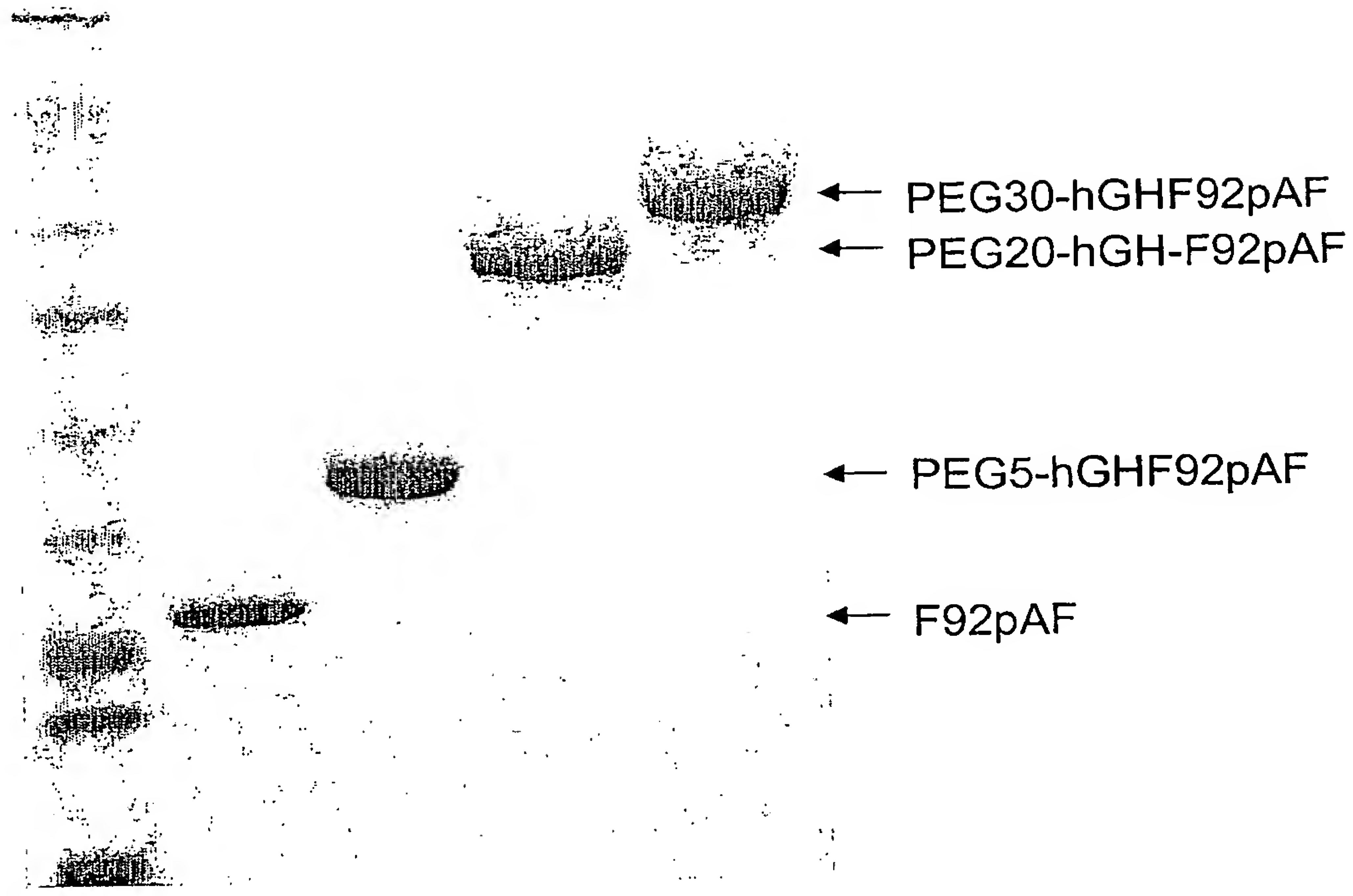
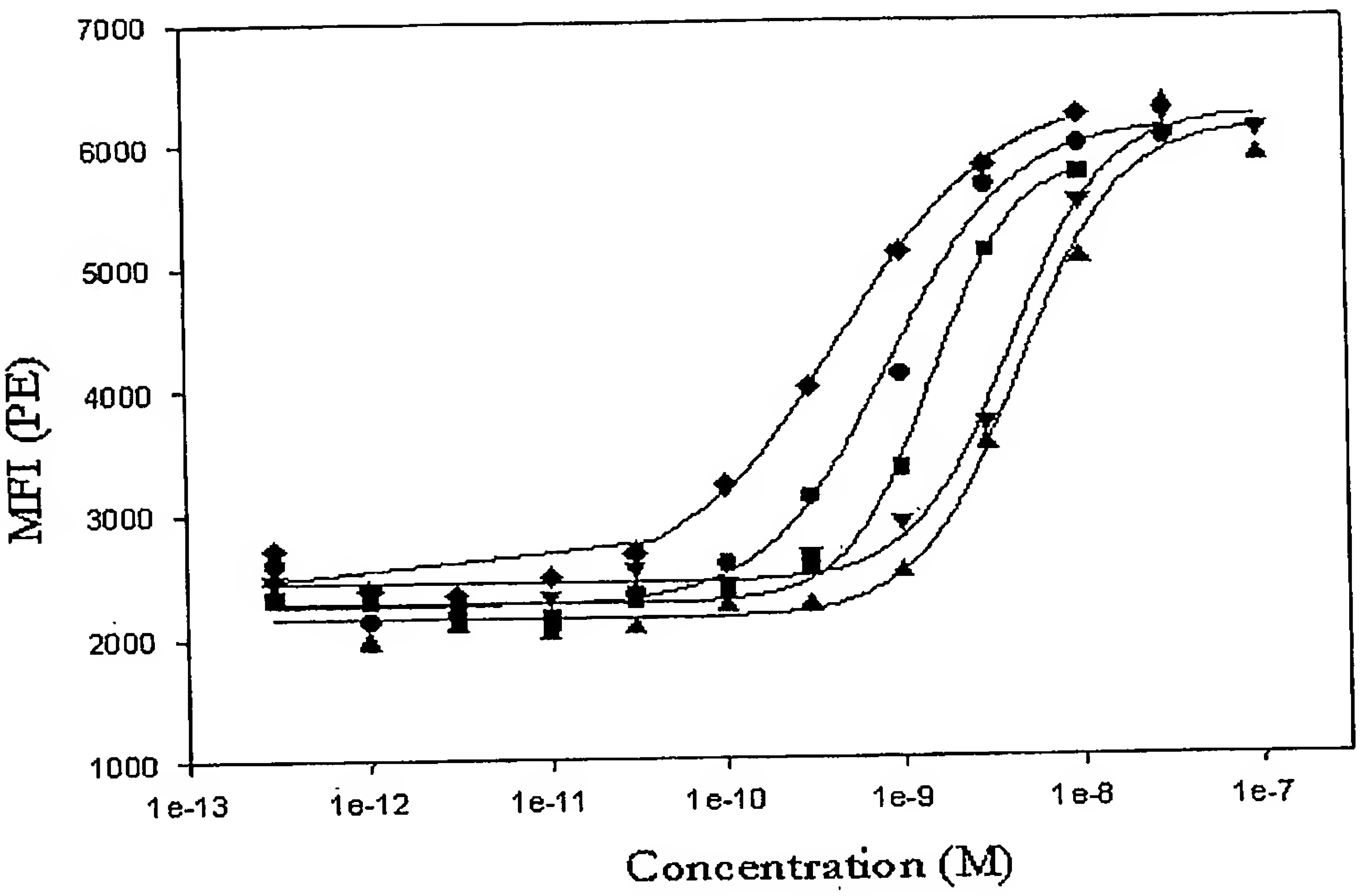

PEG30-hGHF92pAF
PEG20-hGH-F92pAF
PEG5-hGHF92pAF
F92pAF

Figure 9



● (His)F92pAF, EC50 = 0.9 nM

■ (His)F92pAF-5K, EC50 = 1.5 nM

▲ (His)F92pAF-20K, EC50 = 4.6 nM

▼ (His)F92-pAF-30K, EC50 = 4.1 nM

◆ (His)hGH, EC50 = 0.5 nM

Figure 10, Panel A:

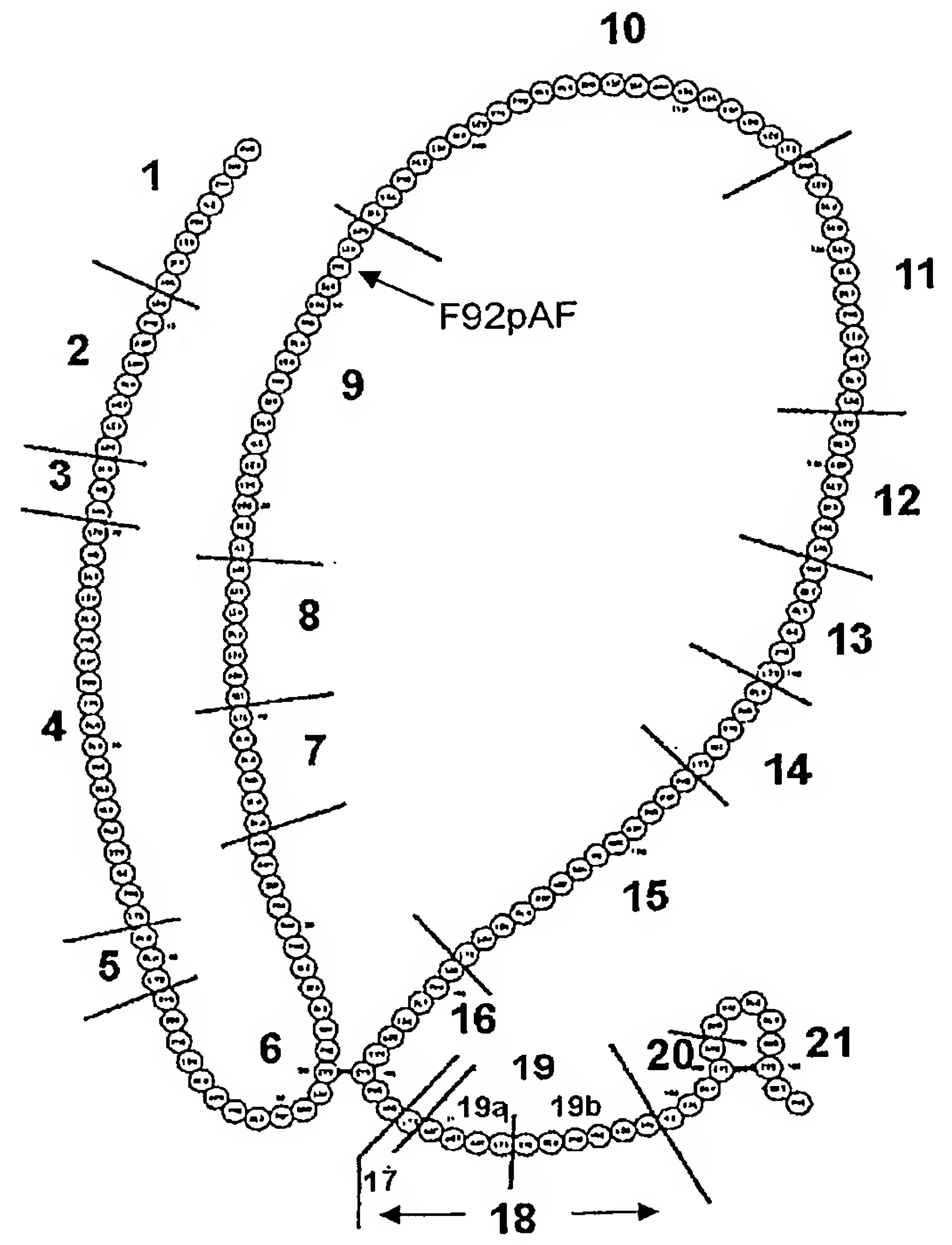
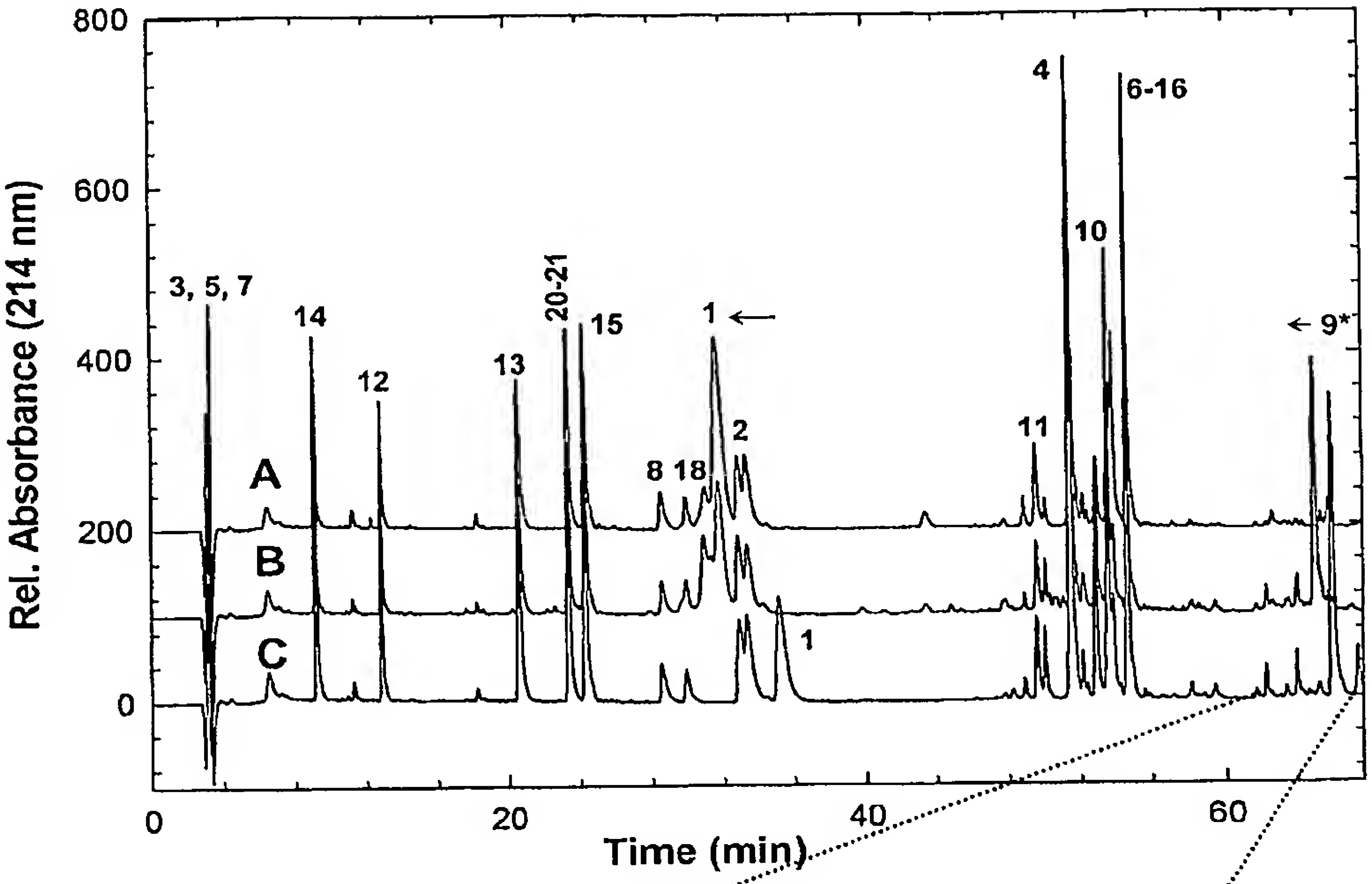

10
1
11
F92pAF
2
9
3
12
8
13
4
7
14
15
5
16
6
19
20
21
19a
19b
17
18

Figure 10, cont.

Panel B:



Panel C:

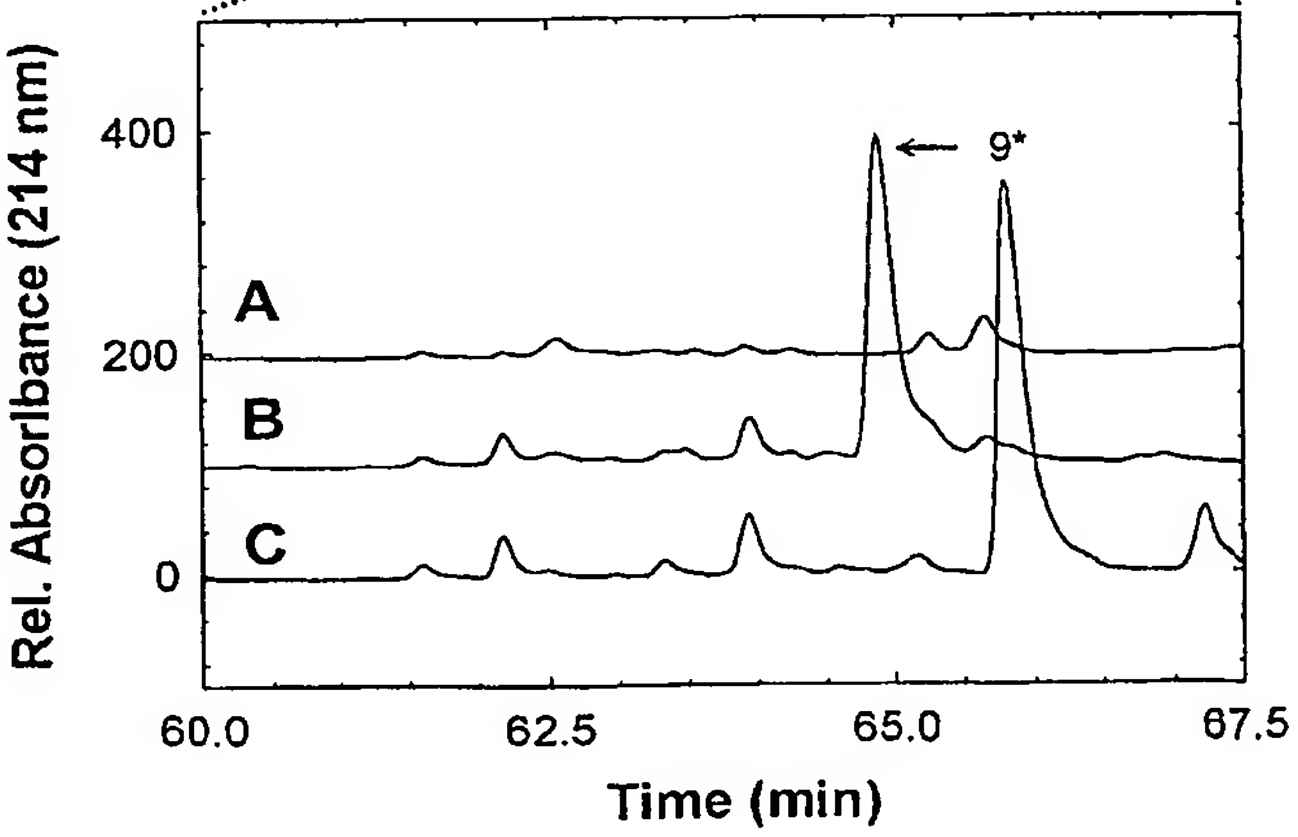


Figure 11

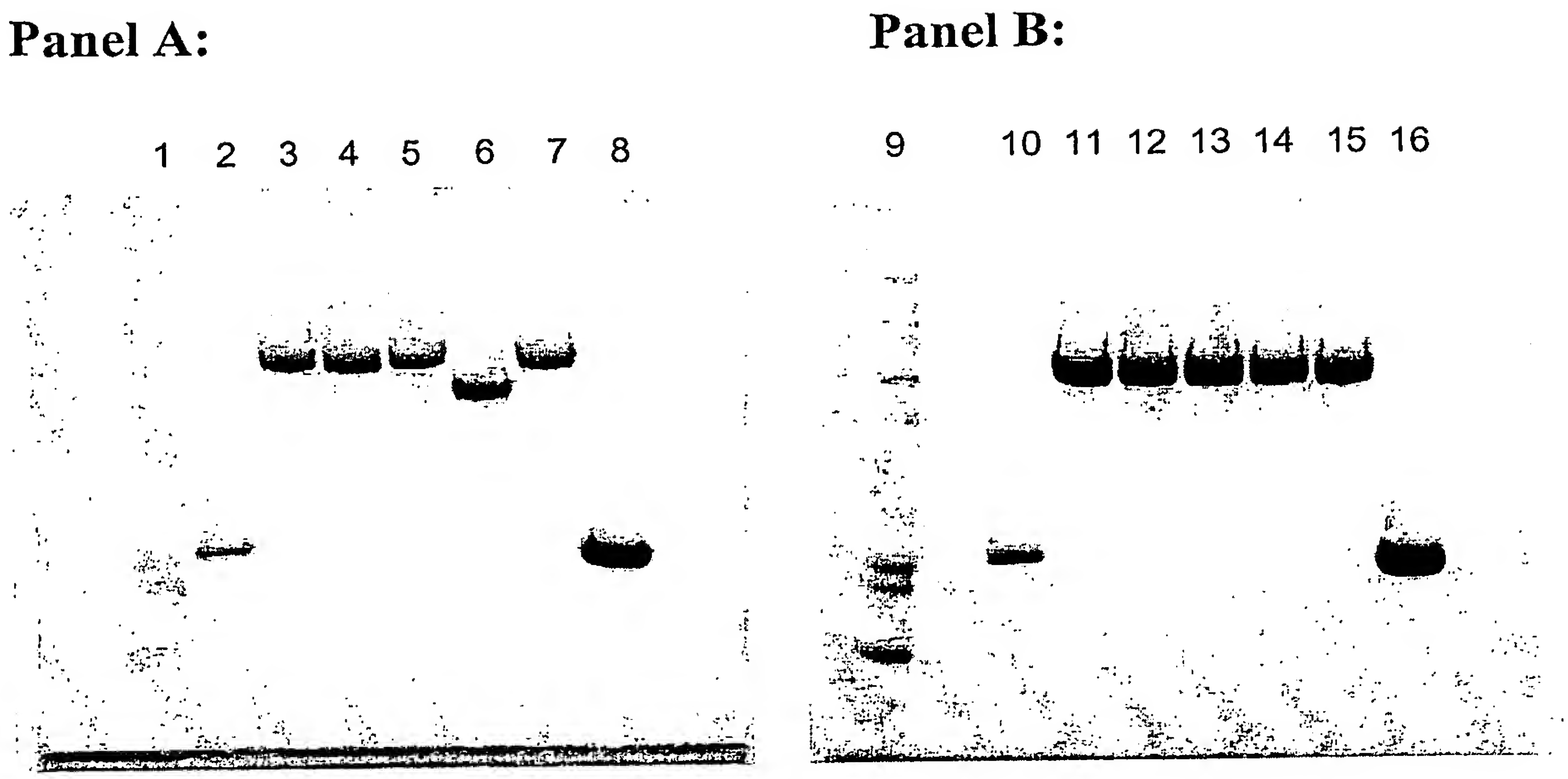

Panel A:
1 2 3 4 5 6 7 8
Panel B:
9 10 11 12 13 14 15 16

Figure 12

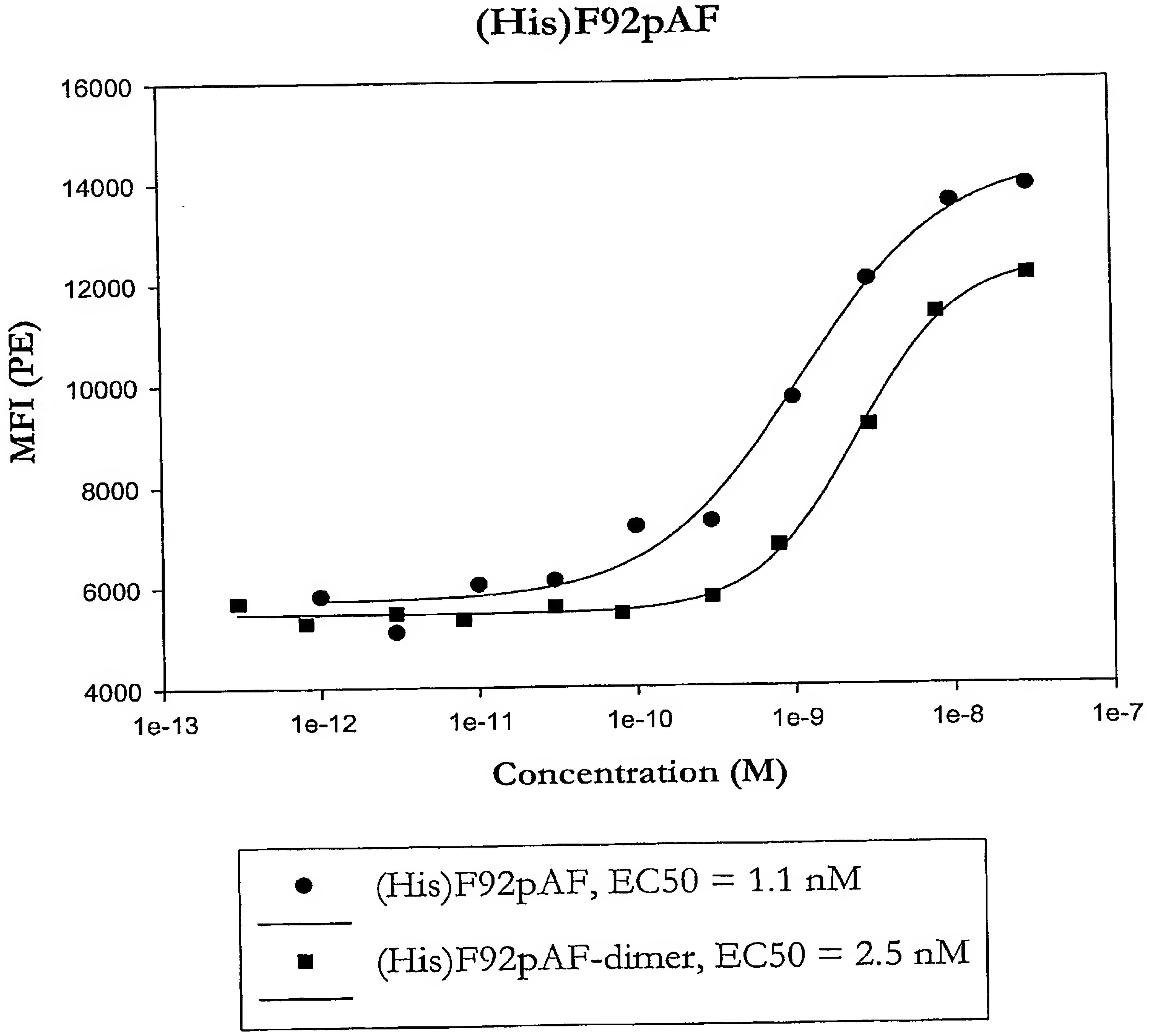

(His)F92pAF
16000
14000
12000
10000
8000
6000
4000
MFI (PE)
1e-13
1e-12
1e-11
1e-10
1e-9
1e-8
1e-7
Concentration (M)
● (His)F92pAF, EC50 = 1.1 nM
■ (His)F92pAF-dimer, EC50 = 2.5 nM

Figure 13, Panel A:

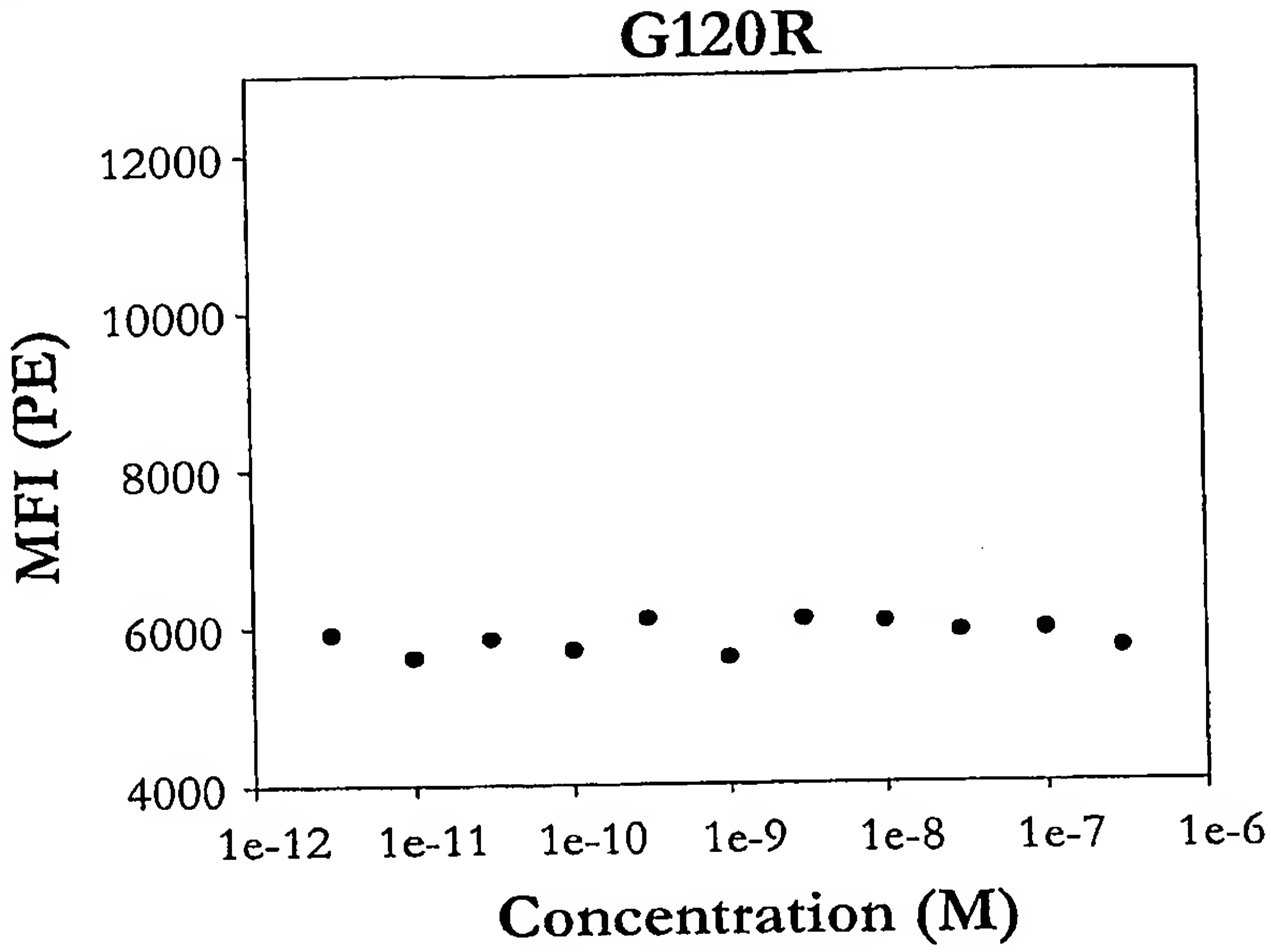


Figure 13, Panel B:

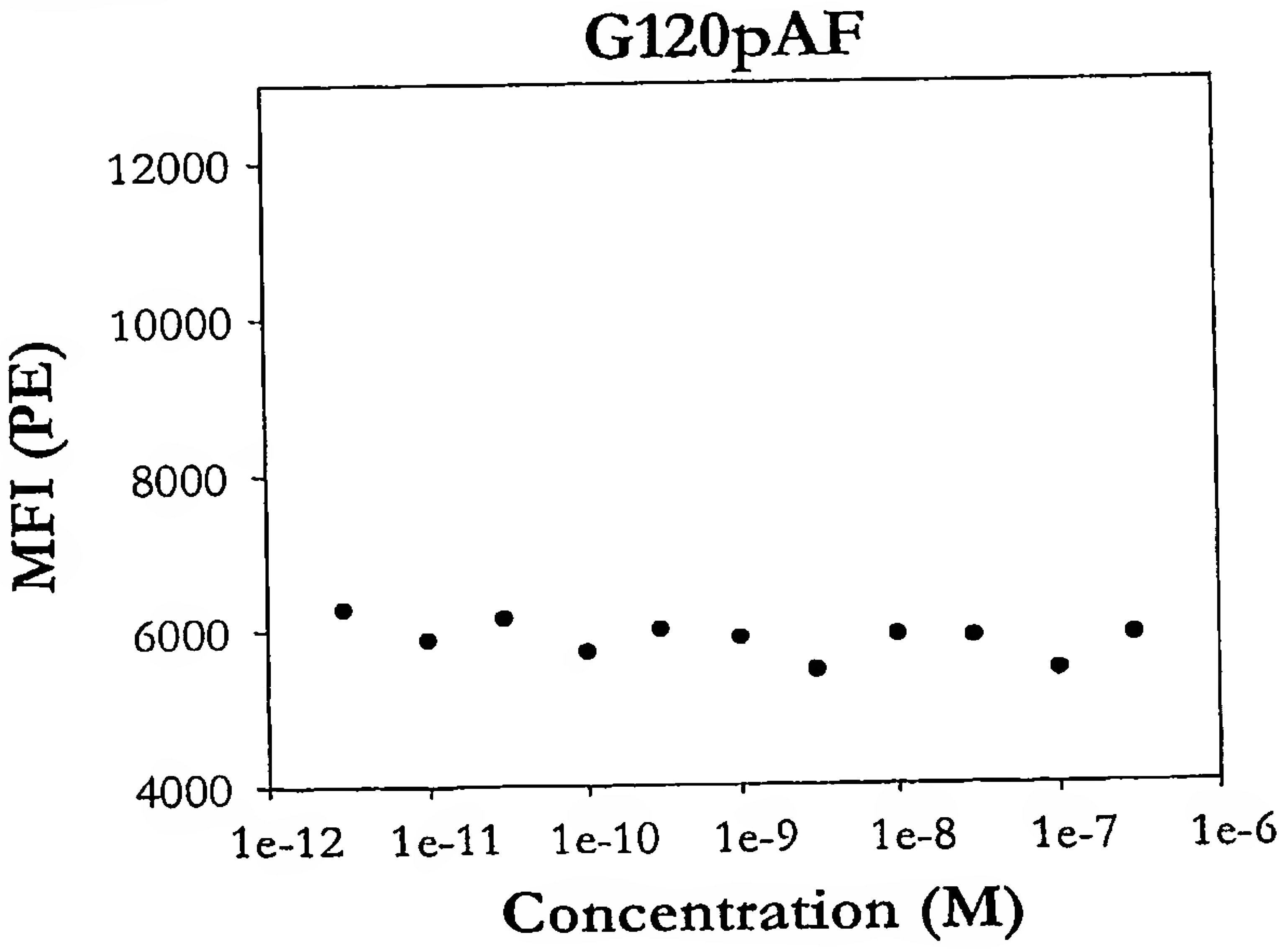


Figure 14

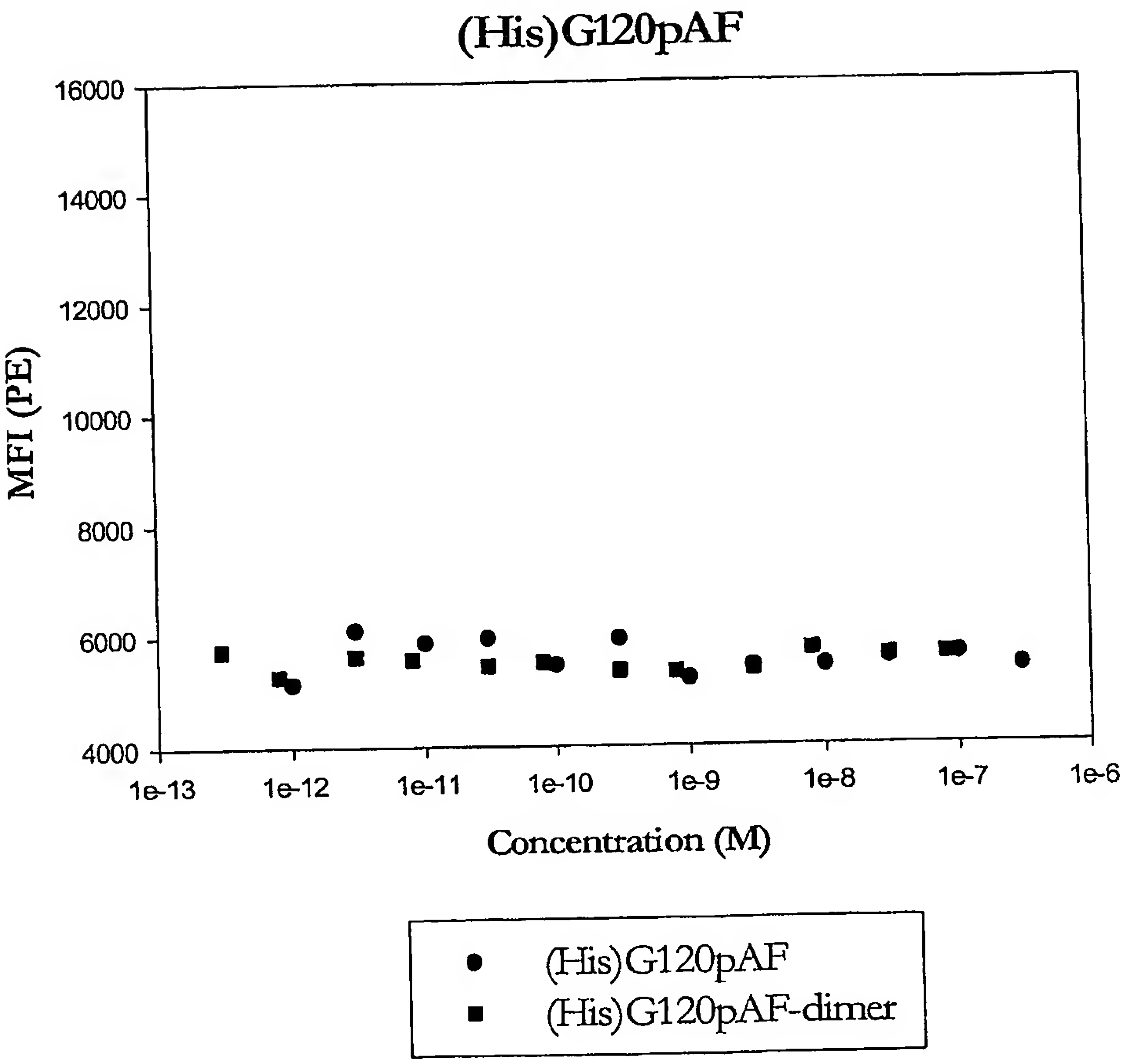

(His)G120pAF
MFI (PE)
16000
14000
12000
10000
8000
6000
4000
1e-13
1e-12
1e-11
1e-10
1e-9
1e-8
1e-7
1e-6
Concentration (M)
● (His)G120pAF
■ (His)G120pAF-dimer

Figure 15

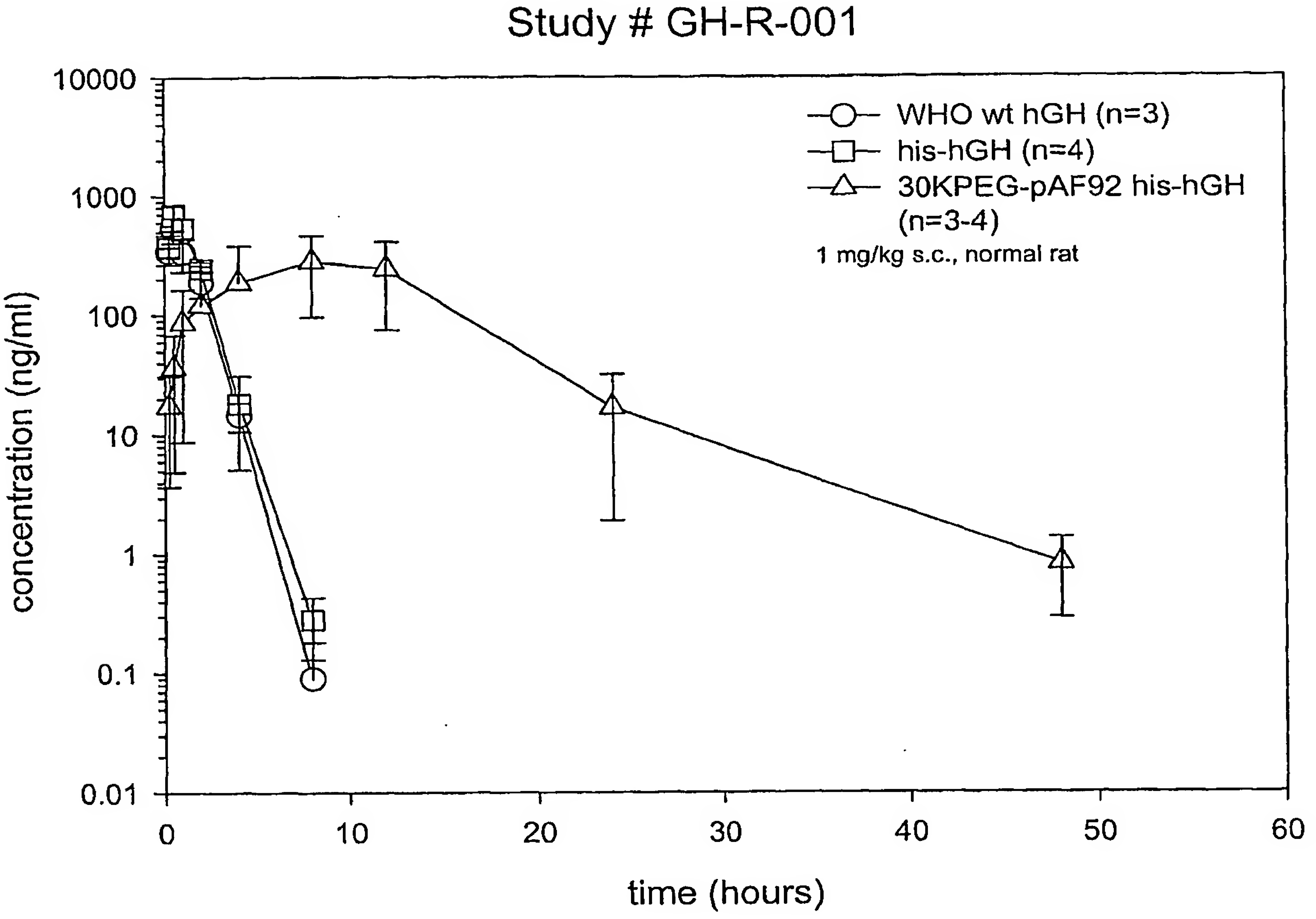

Study # GH-R-001
WHO wt hGH (n=3)
his-hGH (n=4)
30KPEG-pAF92 his-hGH (n=3-4)
1 mg/kg s.c., normal rat
concentration (ng/ml)
10000
1000
100
10
1
0.1
0.01
0
10
20
30
40
50
60
time (hours)

Figure 16

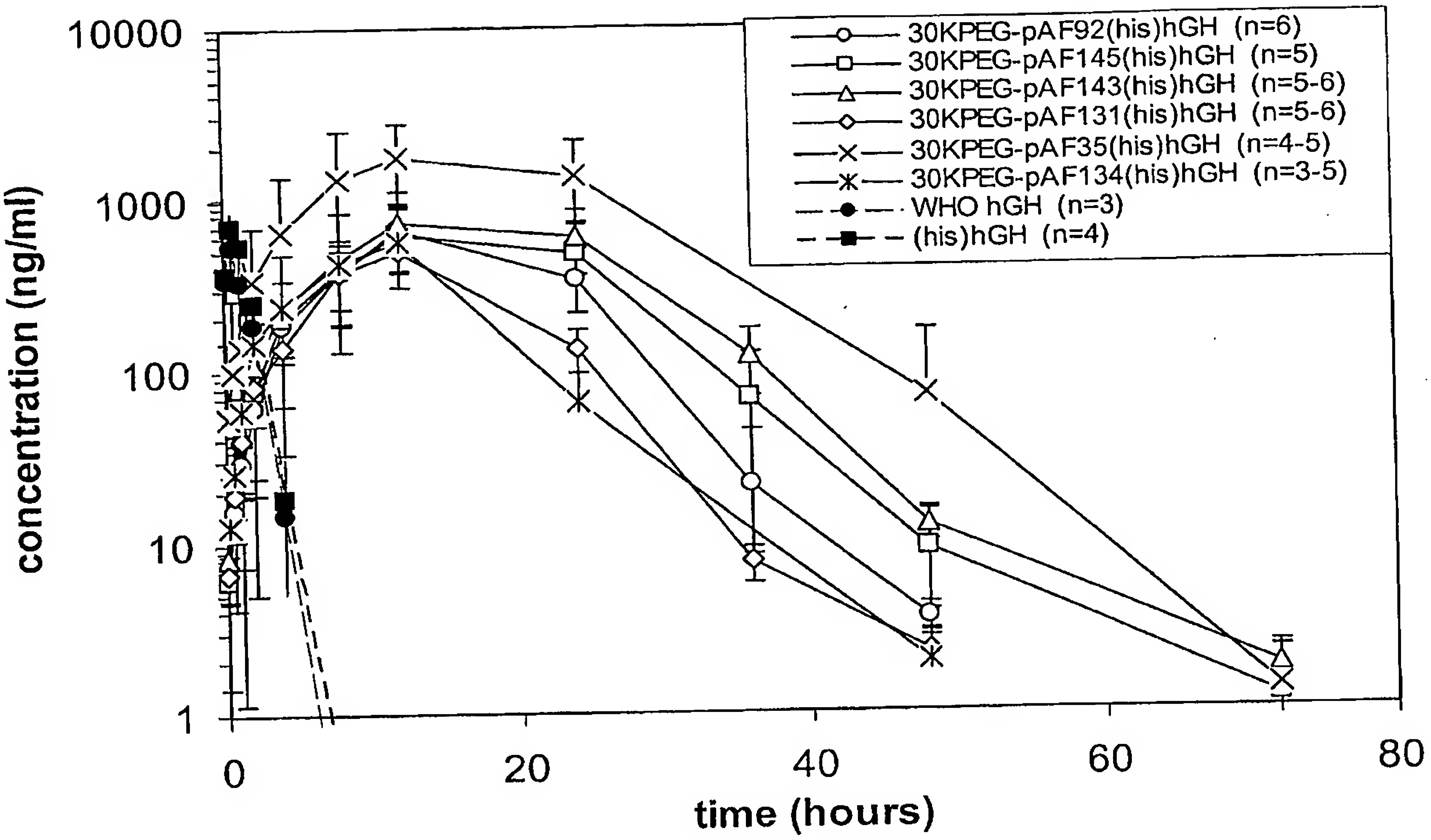

30KPEG-pAF92(his)hGH (n=6)
30KPEG-pAF145(his)hGH (n=5)
30KPEG-pAF143(his)hGH (n=5-6)
30KPEG-pAF131(his)hGH (n=5-6)
30KPEG-pAF35(his)hGH (n=4-5)
30KPEG-pAF134(his)hGH (n=3-5)
WHO hGH (n=3)
(his)hGH (n=4)
10000
1000
100
10
1
concentration (ng/ml)
0
20
40
60
80
time (hours)

Figure 17

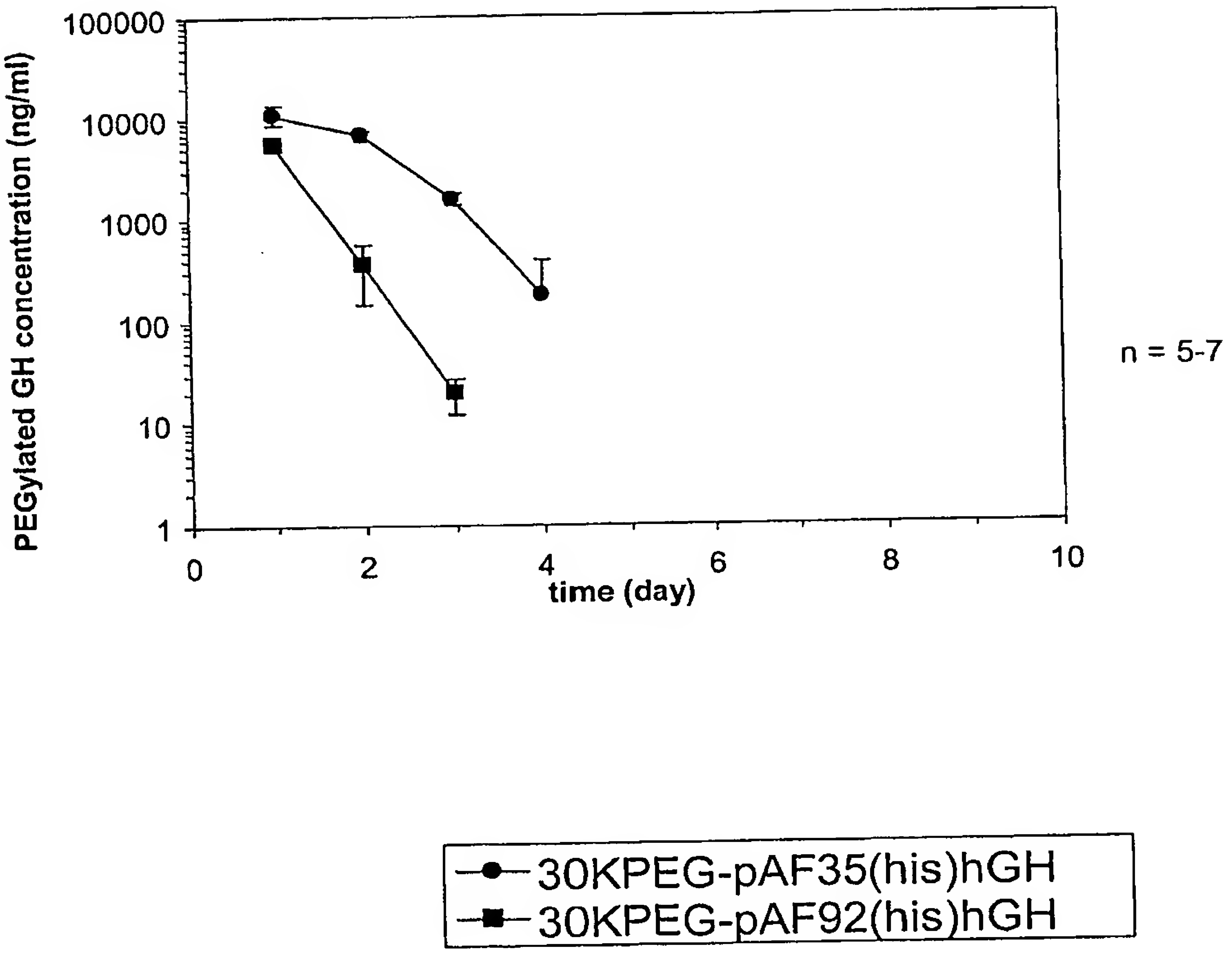

100000
10000
1000
100
10
1
PEGylated GH concentration (ng/ml)
0
2
4
6
8
10
time (day)
n = 5-7
30KPEG-pAF35(his)hGH
30KPEG-pAF92(his)hGH

Figure 18, Panel A

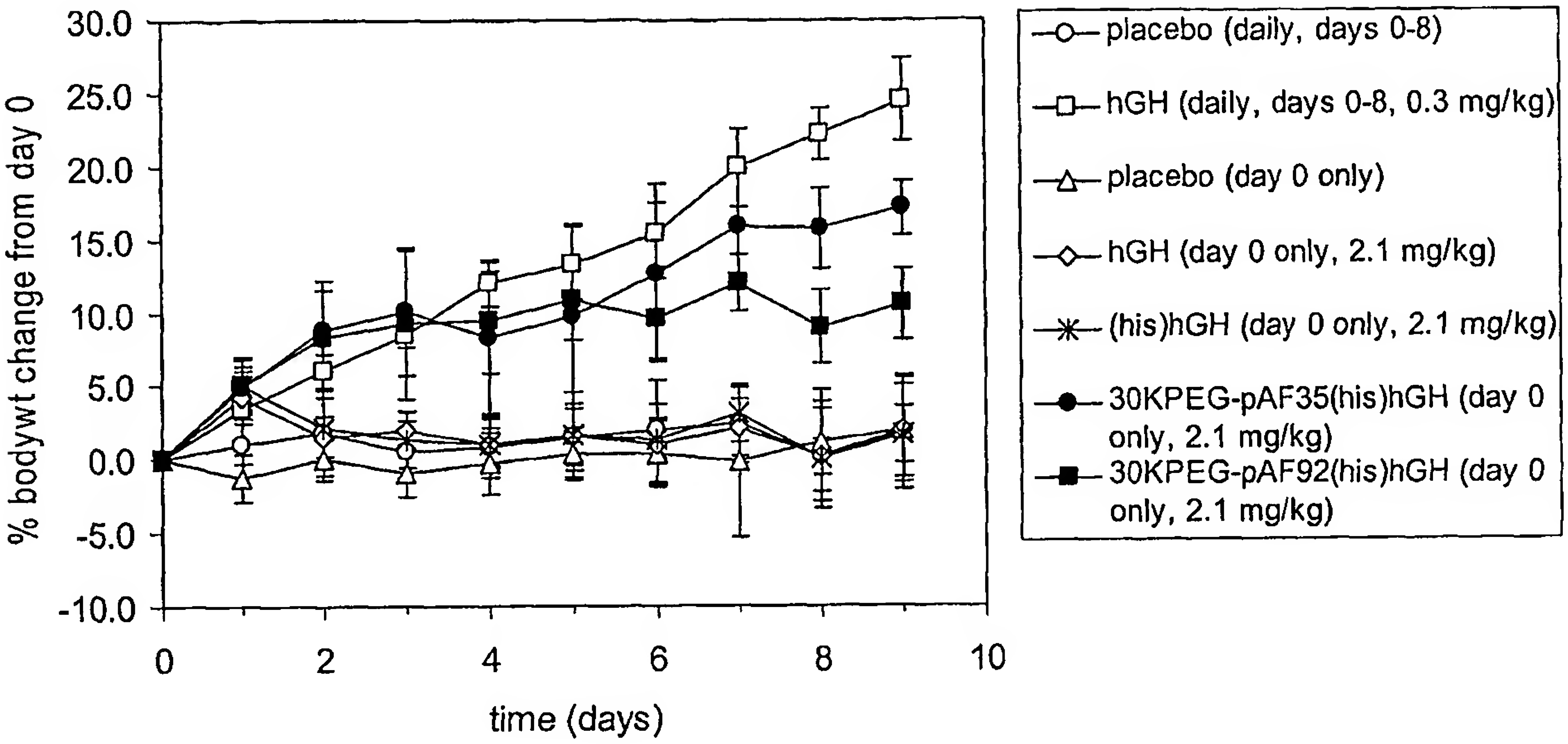


Figure 18, Panel B

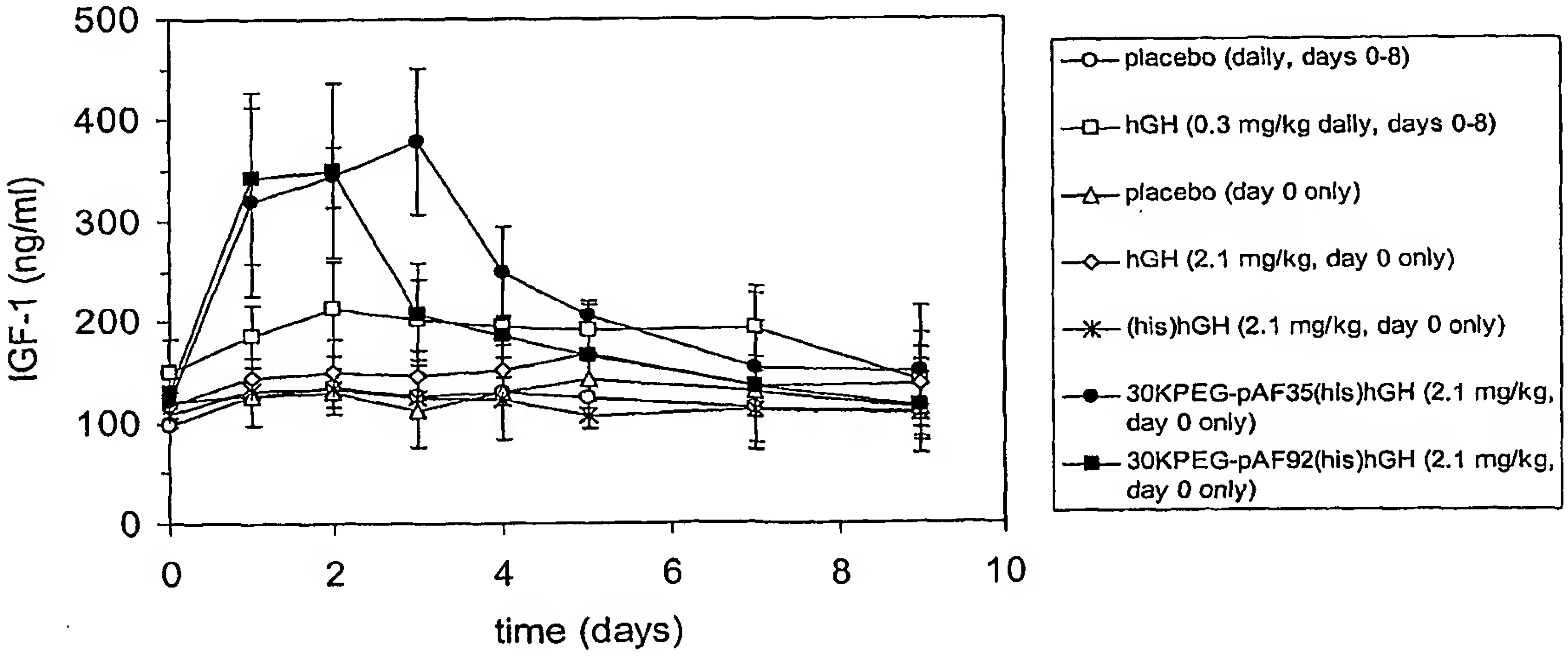


Figure 18, Panel C

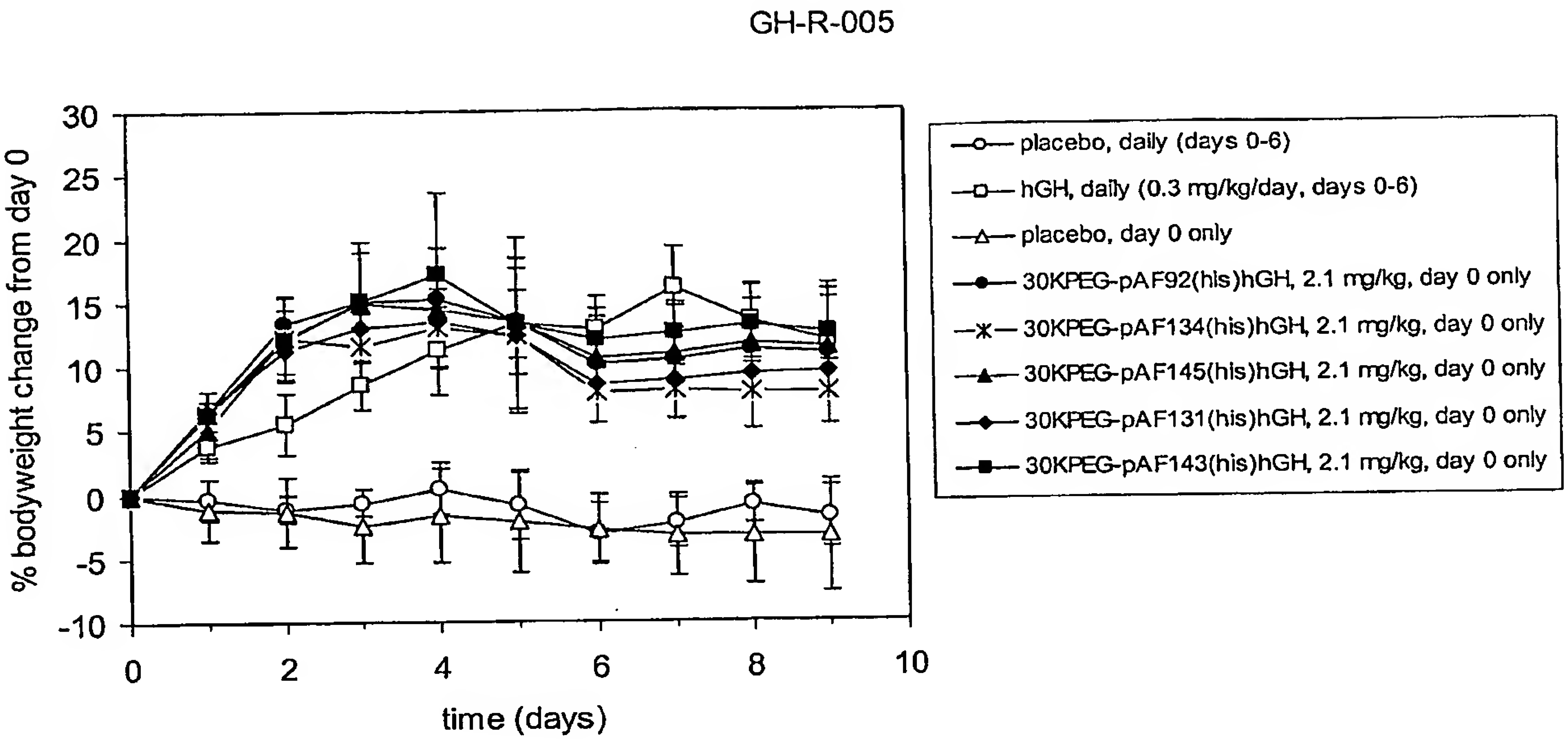
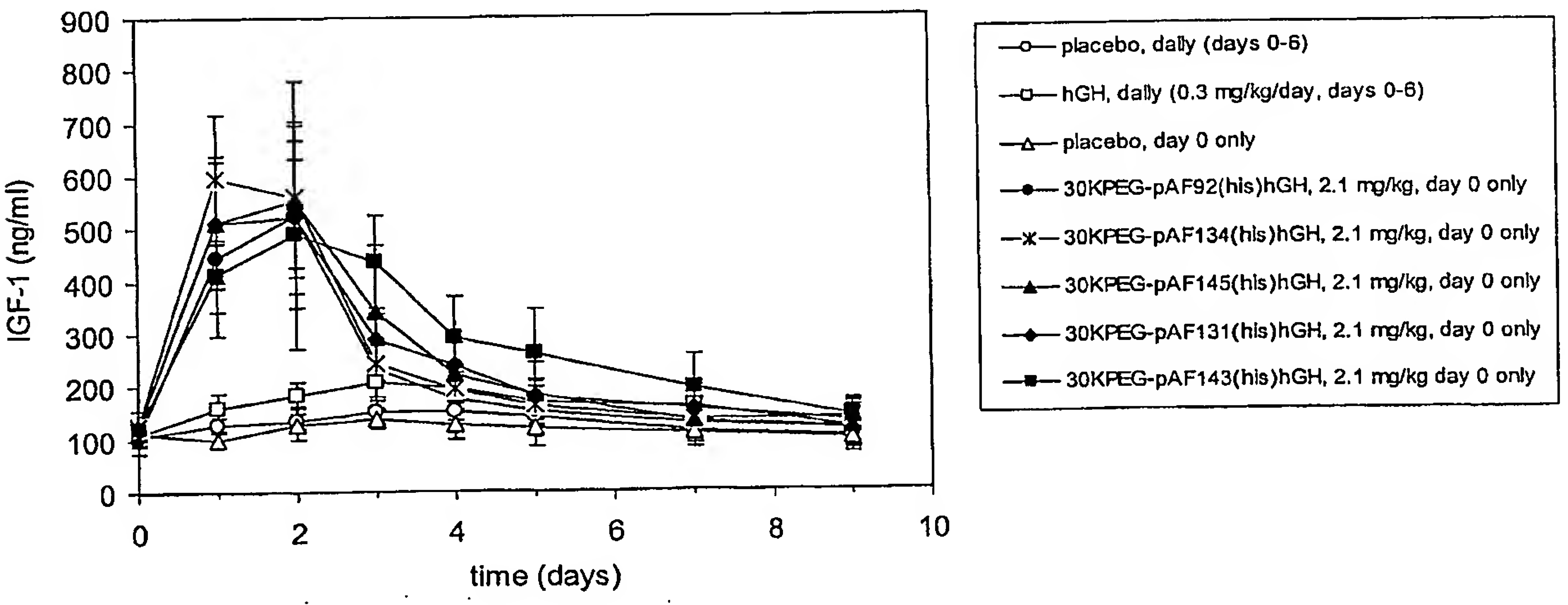
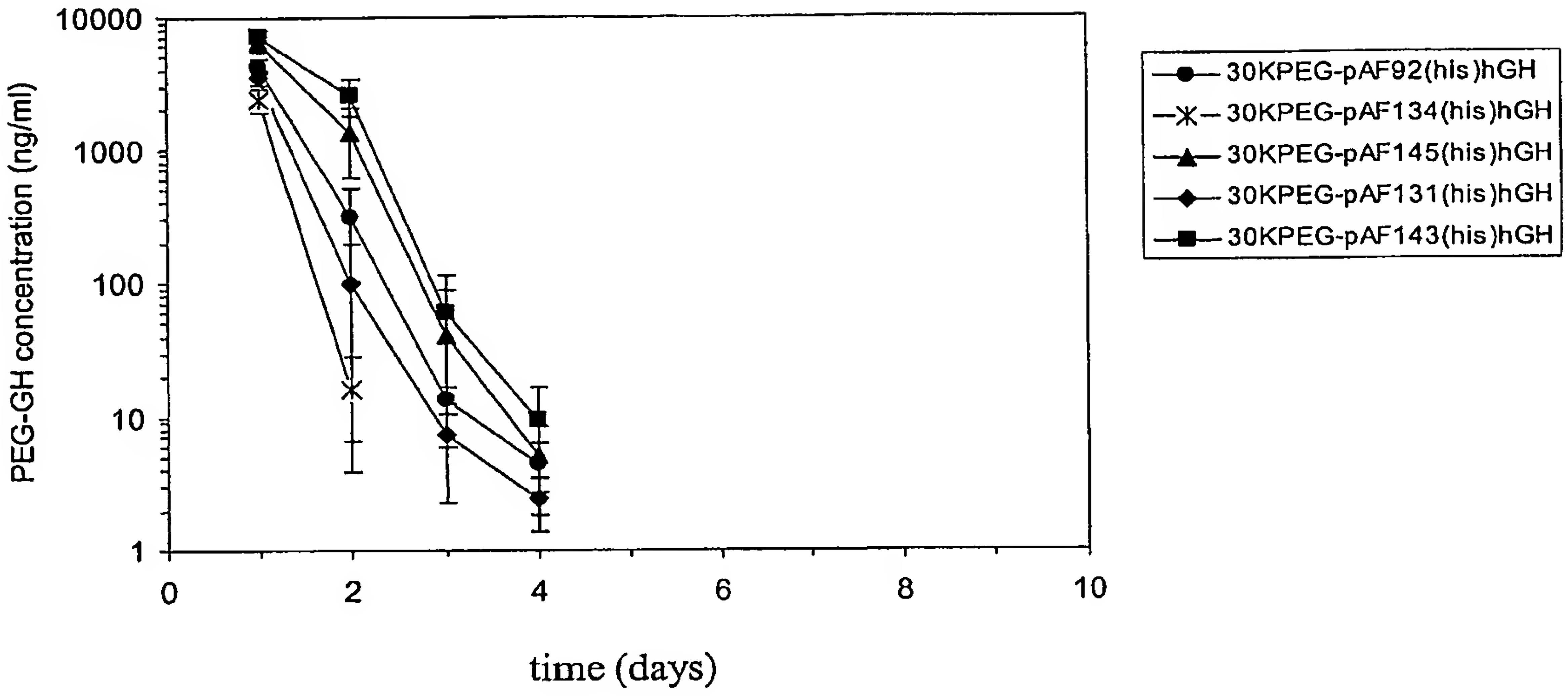


Figure 18, Panel D



Figure 18, Panel E